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Physiologic and genetic comparisons of lysine and leucine distribution in high and low protein corn

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PHYSIOLOGIC AND GENETIC COMPARISONS OF LYSINE AND LEUCINE DISTRIBUTION IN HIGH AND LOW PROTEIN CORN

by

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INTRODUCTION

The relationship between the chemical composition of the vegetative tissue and the seeds of cereal grain plants is not understood. The extent to which organic compounds synthesized in vegetative tissue are modified during and after translocation to the seeds and the efficiency with which different compounds are translocated from the vegetative to the seed tissue are physiological processes. However, these physiological processes are undoubtedly under genetic control. A full understanding of the genetic and physiologic interrelationships of the chemical composition of vegetative and seed tissues is of both fundamental and practical significance. For example, the essential amino acids are more adequately balanced in the protein of the leaves than in the grain of grasses and cereals (Stahmann, 1963). A solution to the problem of amino acid imbalance in corn grain would be valuable in both human and livestock nutrition.

The high and low protein strains of corn, developed at the Illinois Agricultural Experiment Station (Hopkins et al., 1903, and Leng, 1962), provide unique materials for studying amino acid and protein metabolism and translocation in and between the vegetative and seed tissues. That the protein and amino acid characteristics of these strains are under genetic control is well established, and furthermore, the grain protein of the low protein strain is better balanced than that of the high protein strain (Mitchell et al., 1952).

In this investigation two amino acids--lysine which is deficient in the corn grain and leucine which is not--were studied in the vegetative and seed tissues of the high and low protein strains. The specific objectives of the study were:

1. To determine changes in nitrogen, leucine and lysine percentages of different plant parts during the growth cycle.
2. To determine whether percentages of nitrogen, leucine and lysine in the vegetative tissues are related to the percentages of the same compounds in the grain.
3. To determine whether carbon-14 labelled lysine and leucine introduced into the vegetative tissue were differentially incorporated into the grain protein.
4. To observe the metabolic relationships between other amino acids and leucine and lysine.
5. To determine if meaningful biosynthetic patterns in corn can be postulated from long-term experiments with carbon-14 labelled amino acids.

REVIEW OF LITERATURE

The protein of the corn kernel is well described from anatomical, nutritional and chemical standpoints. Feeding studies with laboratory and farm animals have shown that the essential amino acids, lysine and tryptophan, are deficient in corn grain protein. When corn is the sole source of protein in the diet of non-ruminant animals, the ration must be supplemented with lysine or tryptophan for normal growth to occur. Gillespie et al. (1958) and Kocturk et al. (1958) estimated from feeding studies that high (16 per cent) and low protein corn (7 per cent) contained about 30 and 40 per cent, respectively, of optimum quantities of lysine. Osborne and Mendel (1914), Hogan (1917) and Marais and Smuts (1940) also have shown the necessity for supplementing corn grain with lysine and tryptophan.

Early studies based the classification of corn proteins upon solubility differences. Gorham (1821) first described the alcohol soluble protein, zein, that comprised 3.3 per cent of the corn kernel or 40 per cent of the total protein. Showalter and Carr (1922) and Bressani and Mertz (1958) demonstrated that corn varieties were not identical in protein composition. The protein from high protein corn contained a greater proportion of zein than that of low protein corn. Hansen et al. (1946) reported that zein and total protein were related linearly ($r = +.92$). Frey

(1951) also reported high correlations between total protein and zein but showed an exponential relationship which substantiated the work of Showalter and Carr (1922).

The amino acid composition of zein was given by Block and Bolling (1945) as follows:

Amino acid	Per cent of zein
Alanine	11.4
Arginine	1.8
Aspartic acid	5.6
Cystine	1.0
Glutamic acid	26.6
Glycine	0.0
Histidine	1.7
Isoleucine	7.3
Leucine	23.7
Lysine	0.0
Methionine	2.3
Phenylalanine	6.4
Proline	10.4
Serine	7.7
Threonine	3.0
Tyrosine	5.2
Tryptophan	0.1
Valine	3.0

From a nutritional standpoint two factors about zein are of significance: (a) zein comproses 40 or more per cent of corn protein (Bressani and Mertz, 1958), and (b) it contains no lysine and little tryptophan. Four amino acids--alanine, glutamic acid, leucine and proline--comprise 72 per cent of the zein.

Selection experiments designed to modify the protein content of the corn grain have been successful. The classic protein selection experiments were those conducted in Illinois where 61 generations of selection now have been completed (Leng, 1962). In 1896 Hopkins et al. (1903) made protein analyses upon 168 open-pollinated ears from the corn variety "Burr White." The 24 ears with the highest and 12 ears with the lowest protein per cent were used to initiate selection composites for high and low protein, respectively. After 61 generations of selection (Leng, 1962) the mean protein content in high protein lines was approximately twice that of the original population (10.93 per cent to 21.79 per cent), and the mean protein content of low protein lines was less than half that of the original population (10.93 per cent to 4.85 per cent). In each composite, genetic variability was still present after 61 generations of selection.

Unfortunately, the nutritive value of the grain protein in high protein corn is poorer than the grain protein of lower protein corn. Frey (1951) found that tryptophan became a decreasing proportion of the total protein as the protein percentage increased, and Miller et al. (1952) reported similar results for lysine. From an analysis of four United States and four Guatemalan corn strains and teosinte, Bressani and Mertz (1958) showed that leucine and lysine became increasing and decreasing proportions, respectively, of the protein as protein percentage increased. The exception was

the high oil line which combined medium protein, high lysine and low leucine content. They and Frey et al. (1949) proposed that tryptophan and lysine per cent in the whole kernel could be increased by: (a) breeding for larger germ size and/or (b) by increasing the relative percentages of acid and alkali soluble proteins of the endosperm. However, Miller et al., (1950) analyzed ten different single crosses of varying protein content and found little difference between them in amino acid balance.

Zeleny (1935) sampled corn kernels at four stages of maturity and found that zein increased from 2.8 per cent of the nitrogen in immature grain to 42.0 per cent in the mature grain. Concurrently, the water soluble non-protein nitrogen decreased from 41.0 per cent to 4.6 per cent. Globulins and glutelins were synthesized at a uniform rate through all stages. In similar studies Bressani and Conde (1961) reported that zein and non-zein amino acids increased at fast and slow rates, respectively, as the corn grain matured.

To determine changes that had occurred in the Illinois high and low protein lines relative to the original population, Schneider et al. (1952) repeated the experiment of Hopkins et al. (1903) of determining protein content on the kernel parts--germ, endosperm, tip cap and hull--of both lines. In the high protein line there were 44.2 and 4.2 per cent increases in endosperm and germ protein, respectively, whereas the low protein line showed 39.0 and 9.8 per cent decreases in endosperm and nitrogen,

respectively. The difference in protein content of the two lines was only 10 per cent in the germ, but 90 per cent in the endosperm. Germ protein was predominantly water soluble and that of the endosperm predominantly alcohol soluble. They concluded that increasing or decreasing protein in the corn kernel by either fertilization or selection occurs primarily in the endosperm.

Protein content of corn grain can be modified by nitrogen fertilization. MacGregor et al. (1961) found that nitrogen fertilization substantially increased protein percentages, but the proportions of amino acids did not increase uniformly. The levels of some essential amino acids, including lysine, showed little or no increase with increasing protein content. Prince (1954) found a direct relationship between amount of nitrogen applied to the soil and contents of protein, zein, and leucine in the grain. Schneider et al. (1952) agreed that nitrogen fertilization gave higher grain protein content and that zein increased at a disproportionately higher rate. They suggested that the nature of protein increases from selection and fertilization were similar, i.e., the protein increase is due primarily to zein increase.

The protein and amino acid characteristics of corn grain also are found in other members of the grass family. Lawrence et al. (1957) found that the mean lysine content of seed proteins of eight plant families fell in

the following order from high to low: Leguminosae, Cruciferae, Umbelliferae, Liliaceae, Iradaceae, Compositae, Curcubitaceae, and Gramineae.

Lysine per cent in the seed protein of wheat varieties and species was found by Lawrence et al. (1958) to range from 2.64 to 3.84. Hegsted et al. (1954) stated that all feeding studies involving wheat protein have shown it to be deficient in lysine. Rice protein (Kik, 1940) is deficient in lysine, but evidence is confl icting about the level of this amino acid in oat protein (Mitchell and Smuts, 1932, and Marais and Smuts, 1940).

Studies upon protein in the vegetative tissues of plants are not extensive, and generally they have not involved analyses for specific amino acids or proteins. Hoerner and DeTurk (1938) analyzed for total nitrogen in Illinois high and low protein corn plants which had grown for 88 days (until anthesis) in nutrient solutions with varying nitrate levels. At the low nitrate level little difference in nitrogen absorption existed between the two strains; the greatest absorption differential occurred at the medium nitrate level (this corresponded to conditions where the strains were selected) where the high protein strain contained twice as much protein as the low strain.

Hay et al. (1953) studied the degree of transfer of nitrogen compounds from the stalks and leaves to the maturing grain in two Corn Belt hybrids. They concluded that (a) total nitrogen in all vegetative parts of the plant decreased from pollination to maturity; (b) at maturity the grain

contained about two-thirds of the nitrogen found in the entire plant; (c) about two-fifths of the grain nitrogen came from the soil (or roots) after maximum accumulation of nitrogen had occurred in the vegetative tissue; and (d) nitrogen contributed from vegetative plant parts to the grain was 60 per cent from the leaves, 26 per cent from the stalk, 12 per cent from the husk and 2 per cent from the shank.

Hanway (1960) found that 60 per cent of the nitrogen in the mature corn plant was absorbed by the time of anthesis. He also showed that about two-thirds of the plant nitrogen is present in the mature grain. Differences in the per cent nitrogen due to soil fertility were less marked in the stalks than in the leaves or leaf sheaths (Hanway, 1962).

Using two high and two low protein wheat varieties, Seth et al. (1960) found similar protein per cents in the vegetative tissues of all varieties at five different growth stages, except at the milk stage and maturity when the roots of high protein varieties had a lower protein content than those of low protein varieties.

Present knowledge of the movement of nitrogenous compounds in the plant was well stated by Swanson (1959) as follows: "A serious difficulty in nitrogen-transport studies is the uncertainty regarding the identity of the major translocatory compounds of this element. Both inorganic-nitrogen and organic-nitrogen compounds (mostly amino acids and amides) have been identified in the tracherary or xylem sap as well as in phloem

exudate, hence the evidence is presumptive that both forms of nitrogen are transported in each tissue. However, neither the relative amounts translocated as organic nitrogen and inorganic nitrogen nor the relative amounts translocated in phloem or xylem have been accurately established for any species.[1]"

Most of the amino acids, urea and peptides have been found in xylem sap. The quantity of each constituent varies with species (Swanson, 1959). Andreeva (1957) and Ratner and Samoilova (1958) found the nitrogen of "bleeding sap" of young corn plants was mostly in the form of amino acids. In Salix, Kennedy and Mittler (1953) found amino acids in the phloem exudate, and the amount varied with the stage of growth.

Use of radioactive compounds to study the movement of organic metabolites in plants has been restricted primarily to experiments of short duration (less than an hour) designed to elaborate the mechanism of translocation. Nelson and Gorham (1959a and b) found that carbon-14 labelled amino acids introduced into cut petioles of young soybean plants varied in rates of movement and sites of deposition.

Undoubtedly the metabolism and translocation of organic nitrogen is reoriented when grain filling is initiated. Kursanov and Zaprometov (1949)

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[1]Swanson, C. A. Translocation of organic solutes. In Steward, F. C., editor. Plant physiology a treatise. New York, New York, Academic Press. 1959. Volume II. p. 504.

studied the movement of asparagine and certain amino acids in the upper stem parts of excised wheat and rye shoots which had immature ears but no leaves. The nitrogen compounds were absorbed into the shoots by placing the cut ends in amino acid solutions. Most of the amino acids rapidly accumulated in the grain, indicating that the amino acids could be important translocatory compounds.

An extensive series of studies using radioactive amino acids in long-term experiments has been reported by McConnell and his associates. They injected carbon-14 labelled amino acids or amino acid precursors into the uppermost hollow internode of wheat plants at intervals after anthesis and measured the distribution of radioactivity among the chemical components of the mature grain (McConnell and Ramachandran, 1956). Nath and McConnell (1960) recovered as much as 56 per cent of the injected carbon-14 from the seeds. McConnell (1959) showed that radioactive amino acids once formed were rapidly utilized and after being incorporated into storage protein remained substantially unchanged. By administering the tracer compounds at different stages of growth, Bilinski and McConnell (1958) obtained an indication of the biosynthetic sequence in protein formation during grain development. Gliadin predominated, but was formed later than glutenin. The distinctive labelling patterns found for the protein fractions suggested that each protein was biosynthetically independent and that the definable

proteins of the wheat kernels were not artifacts of fractionation from a somewhat continuous spectrum of molecules.

When carbon-14 labelled glutamic acid was injected into the plant, proline and arginine became strongly labelled indicating a close metabolic relationship of these three amino acids (McConnell, 1959). Of the two pathways suggested for the biosynthesis of lysine, i.e., one involving ∝-aminoadipic acid and one involving ∝-∝'-diaminopimelic acid, the latter seems to operate in wheat (Nath and McConnell, 1960, and Finalyson and McConnell, 1960).

MATERIALS AND METHODS

The materials used were derived from samples of Illinois high and low protein corn obtained from the Illinois Experiment Station in 1961.[1] Plants of these seed lots were subsequently inbred for two generations. The progeny from one second-generation inbred plant from each stock (high and low protein) were used for the amino acid and protein studies. Two experiments were run, one in the greenhouse during 1962-63 and one in the field during the summer of 1963. Each will be described separately.

Greenhouse Experiment

On November 1, 36 seeds from the high protein line and 36 seeds from the low protein line were sown, one plant per crock, in two-gallon crocks containing sized gravel. Alternating crocks contained high and low protein plants. Each crock was subirrigated three times daily with a nutrient solution. The quantities of the primary solutions of compounds used to make up the mixed stock solution were as follows:

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[1]Appreciation is expressed to D. E. Alexander, Agronomy Department, University of Illinois, Urbana, Illinois, for supplying the samples of seed of the Illinois high and low protein strains.

Solutions		Ml. used
1 M $MgSO_4$		280
1 M KNO_3		140
1 M KH_2PO_4		140
1 M $Ca(NO_3)_2$		140
Iron 0.5 per cent (Sequestrene 138 was used)		140
Trace elements	gm/liter	140
$MnCl \cdot 4H_2O$	1.86	
$ZnSO_4 \cdot 7H_2O$	0.22	
$CaSO_4 \cdot 2H_2O$	0.105	
H_3BO_3	2.86	
Molybdic acid	.02	

Twenty-five ml. of the stock solution and 3.5 liters of distilled water were added to each crock. Two precautions were taken to assure an adequate nutrient supply for optimum plant growth: (a) the solution for each crock was changed 30 days after planting and subsequently at two-week intervals, and (b) the nitrate levels of the solutions were assayed in the intervening weeks (Bremmer, 1964) and KNO_3 and $Ca(NO_3)_2$ were added to raise the nitrate level to that of the original solution.

It was intended that each plant would be self-pollinated, but due to extreme protandry, selfing was impossible. All high protein plants were sib-pollinated, but the protandry of low protein plants was so extreme it was necessary to pollinate them with pollen from the high protein plants. Fourteen days after pollination six high and five low protein plants were injected with carbon-14 labelled lysine and leucine. The injection date was selected to coincide with the period of high protein synthesis. The specific injection treatments were:

Treatment	Injection treatment		Number of plants
1	25 microcuries	lysine[1]	2 high protein
2	25 "	leucine	2 "
3	50 "	lysine and leucine	2 "
4	25 "	lysine	2 low protein
5	25 "	leucine	1 "
6	50 "	lysine and leucine	2 "

Since the corn stem was solid, a hole had to be made into which the solutions of radioactive materials could be injected. A No. I cork borer was used to remove a section of the internode directly below the ear-bearing node. A 0.1 ml. sample of the appropriate radioactive amino acid solution was injected into the hole with a micropipette. The hole was covered with paraffin and wrapped with plastic tape. In two treatments (3 and 6) the lysine and leucine were injected together to allow a comparison of the metabolism and translocation of both compounds under a constant environmental and genetic system.

The plants were harvested at maturity and the basal 25 kernels from each ear were ground to pass through a 20-mesh screen. In treatments 3 and 6, the second internode above the ear-bearing node was ground similarly. Samples from the two plants receiving the same treatment were combined for analyses.

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[1]Uniformly carbon-14 labeiled L-lysine and L-leucine were obtained from Volk Radio Chemical Company.

Determination of amino acid content

Four-gram samples of the grain and 1.2-gram samples of the internodes were hydrolyzed under reflux in 100 ml. of 6N constant boiling HCl for 24 hours. The excess HCl was distilled off under reduced pressure, and the residue was redissolved in 0.2N sodium citrate buffer (pH 2.2), filtered and made to a 50 ml. volume for the grain and a 25 ml. volume for the internodes. For analyses, 0.5 ml. or 1.0 ml. aliquots of the grain and 2 ml. aliquots of the internode-derived solutions were used.

Quantitative determinations of the concentrations of 16 amino acids and ammonia were made on the aliquots of the hydrolyzed samples using the method of Moore et al. (1958). Block (1960) also describes the method completely. The apparatus used consisted of a long (150 x 0.9 cm.) column containing fraction D of Amberlite IR-120 resin and a short column (15 x 0.9 cm.) containing fraction C of the same resin.[1] Aliquots of the hydrolyzates were added to the columns and eluted with the appropriate buffers. The fractions, each of 1.8 ± .1 ml. (measured volumetrically), were collected in test tubes using an Isco Model A fraction collector. For one determination approximately 240 test tubes were used to collect the fractions from the long column and 120 from the short column. Aliquots of 0.8 ml. were removed from each test tube for future use.

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[1]Both fractions were obtained from the California Biochemical Company.

The quantity of amino acid in each tube, after removal of the aliquot, was determined photometrically (Moore and Stein, 1948, 1954). One ml. of ninhydrin solution was added to each test tube, and the color was developed by placing the tubes in a boiling-water bath for 15 minutes. Next, 5 ml. of a 50:50 (v/v) ethanol-water solution was added to each test tube and the optical density was read using a Bausch and Lomb Spectronic 20 colorimeter. Standard curves constructed from data collected from tubes containing known quantities of leucine were used to convert optical density readings to micromoles of leucine. Since the color values of all amino acids relative to leucine are known (Block, 1960), the concentrations of the other 15 amino acids then could be determined. After the tube with the highest concentration of an amino acid was known, the 0.8 ml. aliquot that had been saved from that tube prior to the ninhydrin treatment was poured onto a planchet and dried. The activity on each planchet was determined by recording the time necessary to give 400 counts on a Model D-47 gas flow counter (Nuclear Chicago Corporation) making the correction for background radiation. Residual radioactivity was removed from the test tubes between determinations by dipping them in cleaning solution (sulfuric acid and potassium dichromate) and washing them with distilled water.

Field Experiment

Seeds of the high and low protein lines were planted on May 30 in adjacent rows 80 feet long and 40 inches apart and subsequently the

seedlings within rows were thinned to a one-foot spacing. Tillers were removed several times during the growing season. The upper ear on each plant was selfed and the second ear, if one developed, was allowed to open pollinate. The frequency of second ears was greater on high protein than on low protein plants.

Four plants were harvested from each line at each of five stages: (I) two weeks before pollination, (II) at pollination, (III) two weeks after pollination, (IV) four weeks after pollination and (V) eight weeks after pollination. Plants harvested at each stage were selected at random from those that were disease free (the material was susceptible to corn smut) and of average size. The plant parts saved for chemical analyses were the leaf sheath, the leaf blade and the internode originating at the first node above the upper ear. In addition, the basal 25 kernels from the upper ear were saved. The four samples, i.e., leaf blade, leaf sheath, internode and seeds, were prepared for analysis by grinding to pass through a 20-mesh screen. The remaining parts of each plant were dried at 43°C and weighed.

Nitrogen determinations

Determinations of ammonium nitrogen were made by the microkjeldahl method described by Perrin (1953). One hundred mg. samples were analyzed using a selenium catalyst.

Lysine and leucine determinations

Because no major differences in the nitrogen content of the leaf sheath of the high and low lines were noted, lysine and leucine determinations were made only on the leaf blade, internode and grain. The samples were prepared for the microbiological assay by hydrolyzing with 10-15 ml. of 2N HCl in a covered 100-ml. beaker in an autoclave at 15 pounds pressure and 121°C for 12 hours. The excess acid was evaporated to near dryness on a steam plate and to complete dryness in an oven at 37°C. Next, 10 ml. of water were added to the residue, the pH adjusted to 4.0 and the solution was filtered through a fritted glass filter. The filtration was to remove an "active" substance in the humin which causes errors in lysine determinations (Horn et al., 1953). The pH of the filtered samples was adjusted to 6.8 with NaOH and made up to 50 ml. volume from which aliquots were taken for lysine analyses. Five to 25 ml. aliquots also were taken and made to 100 ml. volume for leucine analysis.

The amino acid analyses were made by a microbiological assay described by Williams (1955) and Steel et al. (1949) using Leuconostoc mesenteriodes strain P-60. Standard prepared media from Difco Laboratories were used for the assays. Duplicate tubes of four levels of each sample were assayed. Standard curves were obtained using nine levels of leucine and ten levels of lysine run in quadruplicate. Lactic acid

produced in each tube was titrated to neutrality with .075N NaOH using a pH meter, and the titration values for the sample tubes were converted to concentration of lysine and leucine by comparison with standard curves.

RESULTS

Greenhouse Experiment

The per cents of the 16 amino acids in the grain and internode of the high and low protein lines are given in Tables 1 and 2, respectively. The

Table 1. The contents of 16 amino acids and ammonia in the grain of high and low protein corn expressed as a per cent of grain dry weight, and the ratio of the per cents of each amino acid in the high to low protein strains

Amino acid	High protein	Low protein	Per cent in high / Per cent in low
Alanine	1.51	.48	3.17
Arginine	.62	.28	2.21
Aspartic acid	1.33	.45	2.94
Glutamic acid	3.58	.84	4.27
Glycine	.51	.26	1.99
Histidine	.47	.15	3.20
Isoleucine	.65	.26	2.52
Leucine	2.50	.59	4.26
Lysine	.37	.24	1.56
Methionine	.22	.09	2.20
Phenylalanine	.87	.30	2.92
Proline	2.11	.82	2.57
Serine	.83	.25	3.37
Threonine	.60	.22	3.73
Tyrosine	.58	.18	3.17
Valine	1.47	.55	2.64
NH_3	.44	.12	3.68

percentages were calculated using the following formula:

amino acid (per cent of tissue dry weight) =

$$\frac{\text{(leucine equivalents)(molecular weight of amino acid)(100)}}{\text{(color yield)(sample size)}}$$

Cystine could not be detected, either because it was present in such small quantities or, because of extreme sensitivity to the buffer pH, it came off the column with alanine.

Table 2. The contents of 16 amino acids and ammonia in the internodes of high and low protein corn expressed as a per cent of the tissue dry weight

Amino acid	High protein	Low protein
Alanine	.18	.18
Arginine	.10	.12
Aspartic acid	.30	.18
Glutamic acid	.29	.25
Glycine	.17	.11
Histidine	.06	.04
Isoleucine	.10	.04
Leucine	.16	.09
Lysine	.10	.12
Methionine	.04	--
Phenylalanine	.08	.06
Proline	.24	.26
Serine	.13	.15
Threonine	.12	.11
Tyrosine	.06	.08
Valine	.26	.15
NH_3	.12	.04

All of the amino acid percentages were greater in the high than in the low protein grain (Table 1). However, the ratios of the per cents of the individual amino acids between high and low protein corn grain varied considerably. The ratios of glutamic acid and leucine were greater than 4 to 1, whereas the lysine ratio was only 1.5 to 1. The ratios for the other amino acids varied between 2 to 1 and 4 to 1.

The amino acid per cents for the internode tissues were lower than those for the grain, but in general, there were no major differences of the amino acid percentages in the internode tissue between the high and low protein lines (Table 2).

The radioactivity was determined for each amino acid on only one tube, i.e., the one containing the greatest quantity of the amino acid. By knowing the amount of the amino acid present in the tube which was counted, the specific activity could be calculated as counts per minute per milligram. The specific activity then was multiplied by the total quantity of the amino acid in all of the tubes to give total activity.

Intuitively, total activity would seem to be a better measure for comparisons than specific activity because the carbon-14 labelled amino acids were injected into the plants only once, and the specific activity is affected by the amount of the amino acid present. However, both tend to show the same trends (Tables 3, 4 and 5).

Table 3. Specific and total activities of the 16 amino acids isolated from the grain of high protein plants injected with uniformly carbon-14 labelled lysine and leucine

Amino acid	Injection treatment					
	(1)[a] lysine-C^{14}		(2)[a] leucine-C^{14}		(3)[a] lysine-C^{14} and leucine-C^{14}	
	Activity		Activity		Activity	
	Specific[b]	Total[c]	Specific[b]	Total[c]	Specific[b]	Total[c]
Alanine						
Arginine	405	181			497	143
Aspartic acid						
Glutamic acid	50	123	31	99	30	44
Glycine					233	52
Histidine						
Isoleucine	42	19	3394	1940	161	44
Leucine	20	35	1328	2770	749	801
Lysine						
Methionine			129	27		
Phenylalanine						
Proline	56	89	62	114	44	36
Serine						
Threonine			143	76	123	29
Tyrosine						
Valine	15	15	64	85		

[a]For treatments 1 and 2, 80 mg. samples were analyzed and for treatment 3, a 40 mg. sample.

[b]Counts per minute per mg.

[c]Counts per minute.

In all plants where carbon-14 labelled leucine was injected (treatments 2, 3, 5 and 6), radioactive leucine was isolated from the grain. When labelled leucine was injected into high protein plants

Table 4. Specific and total activities of the 16 amino acids isolated from the grain of low protein plants injected with uniformly carbon-14 labelled lysine and leucine

Amino acid	Injection treatment					
	(4)[a] lysine-C^{14}		(5)[a] leucine-C^{14}		(6)[a] lysine-C^{14} and leucine-C^{14}	
	Activity		Activity		Activity	
	Specific[b]	Total[c]	Specific[b]	Total[c]	Specific[b]	Total[c]
Alanine	451	59				
Arginine						
Aspartic acid					71	14
Glutamic acid						
Glycine						
Histidine						
Isoleucine			1894	226	1622	204
Leucine			833	187	930	213
Lysine	4184	367			4271	366
Methionine						
Phenylalanine						
Proline	165	59				
Serine						
Threonine						
Tyrosine						
Valine			294	79		

[a]Forty mg. samples were analyzed.

[b]Counts per minute per mg.

[c]Counts per minute.

(treatment 2) over 90 per cent of the total activity found in the 16 amino acids of the grain was present in leucine and isoleucine. Small amounts of activity also were recovered in glutamic acid, methionine, proline, threonine and valine. When carbon-14 labelled lysine was injected into

Table 5. Specific and total activities of the 16 amino acids isolated from the internodes of high and low protein plants injected with both uniformly carbon-14 labelled lysine and leucine

Amino acid	Injection treatment (3)[a] lysine-C^{14} and leucine-C^{14} Activity Specific[b]	Total[c]	(6)[a] lysine-C^{14} and leucine-C^{14} Activity Specific[b]	Total[c]
Alanine				
Arginine			1209	152
Aspartic acid			260	46
Glutamic acid			259	68
Glycine				
Histidine			1627	68
Isoleucine	779	71	3482	132
Leucine	1398	195	2137	209
Lysine			4774	609
Methionine				
Phenylalanine				
Proline			88	24
Serine				
Threonine			211	25
Tyrosine				
Valine				

[a]An 89 mg. sample was analyzed for treatment 3 and a 105 mg. sample for treatment 6.

[b]Counts per minute per mg.

[c]Counts per minute.

high protein plants (treatment I) small amounts of radioactivity were recovered from the grain in arginine, glutamic acid, isoleucine, leucine, proline and valine, but no recoverable activity was found in the lysine

fraction. In treatment 3 (labelled leucine and lysine injected into the same high protein plants), most of the activity recovered from the grain was in leucine while none was recovered in the lysine fraction. However, radioactive arginine, glutamic acid, glycine, isoleucine, proline and threonine were found also.

Since no radioactive lysine was recovered in the grain from high protein plants injected with labelled lysine, it appears that this amino acid did not move intact from the vegetative to the grain tissue. This apparent inability of lysine movement could account in part for the deficiency of this amino acid in the corn grain.

When carbon-14 labelled lysine was injected into low protein corn plants (treatment 4) some radioactive alanine and proline were found in the grain but most of the activity recovered was in lysine. With the injection of labelled leucine into a low protein corn plant (treatment 5), radioactivity was recovered in isoleucine, leucine and valine. When both labelled amino acids were injected into the low protein corn plants (treatment 6), four amino acids with radioactivity were found in the grain--isoleucine, leucine, lysine and aspartic acid.

The radioactivity recovered in the lysine and leucine from the internodes (Table 5) of high and low protein plants injected with both amino acids was similar to the pattern established in the grain (treatments 3 and 6). When both labelled amino acids were injected into high protein plants,

activity was recovered in only isoleucine and leucine. With the injection of both amino acids into low protein plants, on the other hand, both radioactive leucine and lysine were recovered from the internode tissue. Activity was found also in arginine, aspartic acid, glutamic acid, histidine, isoleucine, proline and threonine.

Thus, the largest and most significant contrast between the two strains was found where labelled lysine was injected. Large amounts of radioactive lysine were found in the grain of the low protein plants, but no activity was recovered in lysine from the grain of high protein plants. In treatments 3 and 6, where the internodes were analyzed also, the labelling pattern for the two amino acids was similar to the grain. This suggests that the basis for the lysine deficiency of high protein grain might be found in the vegetative tissue. Either enzymes responsible for the catabolism of lysine were excessively active in the vegetative tissue or lysine was not translocated from the area of injection. With either explanation the defect most likely is established with the inception of grain filling.

No pathway for direct synthesis of isoleucine from leucine (Fruton and Simmonds, 1958) has been postulated. However, the high specific and total activity of isoleucine whenever leucine was injected indicate that such a pathway could exist in corn.

The method used to determine radioactivities of the amino acid fractions had three limitations: (1) only a fraction of each amino acid was actually counted; (2) a ten per cent error was possible with the volumetric device used to collect fractions and (3) only 400 counts were made on a sample. Thus, rather large errors could be associated with the amino acids with low activities. Direct comparisons of amino acids other than leucine, isoleucine and lysine would be confounded with these error sources. However, there are indications of possible metabolic relationships. In the high protein line, activity was recovered consistently in glutamic acid and proline when either radioactive lysine or leucine were injected, and labelled arginine was recovered when radioactive lysine was injected. Somewhat similar patterns of labelling were observed in wheat by McConnell (1959) and Nath and McConnell (1960).

It is difficult to draw any conclusion from the recovery of activity in the other amino acids because of a lack of consistency across treatments. For example, radioactive methionine was recovered in treatment 2 where labelled leucine was injected but not in treatment 3 where labelled leucine also was injected.

Field Experiment

Weights

There was considerable variation in the weights of plant parts used for chemical analyses (Table 6). However, the high and low protein lines

were similar for total plant weight and relative proportions of leaves and grain (Table 7). Thus, chemical percentages are adequate indicators of

Table 6. Mean weights in grams of leaves, leaf sheaths, internodes and 25 kernels of high and low protein corn at five stages of harvest

Harvest stage	Leaf	Leaf sheath	Internode	Grain--25 kernels
		Low protein		
I	1.79			
II	2.70	1.68	2.15	
III	2.87	1.80	1.89	1.94
IV	3.26	2.14	2.29	3.81
V	2.97	1.94	2.72	6.73
		High protein		
I	2.78			
II	4.30	2.00	2.41	
III	3.95	1.75	2.53	2.56
IV	4.64	2.17	3.91	2.94
V	1.95	1.80	2.39	4.39

chemical differences because the percentages are not confounded by differential weights. The lower weights at Stage V were due to losses caused by insects, wind and rain.

Nitrogen, leucine and lysine content

Separate analyses of variance were calculated for each chemical constituent measured in the leaves, leaf sheaths, internodes and grain. A replication consisted of the parts of one plant from each harvest stage.

Table 7. Mean weights in grams of leaves, grain and whole plants of high and low protein corn at five stages of harvest

Harvest stage	Leaves	Grain	Whole plant (including grain and leaves)
		Low protein	
I	20.0		43.8
II	25.7		109.3
III	26.1	26.7	191.8
IV	28.9	58.3	227.0
V	21.2	101.0	252.8
		High protein	
I	27.0		49.4
II	33.6		117.7
III	29.7	29.1	174.5
IV	33.4	50.2	227.7
V	17.5	92.9	239.5

Since the plants were assigned to replications after all harvests were completed, the sum of squares for replications in the analyses of variance reflect variation between different runs of the chemical analyses.

The nitrogen percentages at the various stages of harvest were significantly different in the leaves, leaf sheaths and internodes of both lines and for the grain of the low protein line (Table 8). In general, nitrogen content decreased in all plant parts from Stages I to V (Table 9). The leaf sheaths had much lower nitrogen percentages than did the leaves in both corn lines, but there was a marked difference in the nitrogen percentage of the internodes of the high and low protein lines. In the high

Table 8. Mean squares from analyses of variance of nitrogen percentages of leaves, leaf sheaths, internodes and grain of high and low protein corn harvested at five stages

Source of variation	Degrees of freedom	Mean squares	
		Low protein	High protein
Leaves			
Replications	3	.0858	.0196
Stages	4	.9491**	3.1916**
Error	12	.0214	.0309
Leaf sheaths			
Replications	3	.0019	.0219
Stages	3	.0268*	.3101**
Error	9	.0040	.0283
Internode			
Replications	3	.0113	.0334
Stages	3	.1953**	2.9814**
Error	9	.0085	.1570
Grain			
Replications	3	.0180	.0532
Stages	2	.5422**	.1718
Error	6	.0058	.0623

*Significant at the .05 level of probability, herein throughout.

**Significant at the .01 level of probability, herein throughout.

protein line, the internode and leaf nitrogen percentages were similar, whereas in the low protein line the internodes contained only from one-quarter to one-third as much nitrogen as the leaves. At maturity the grain of the high protein line had a nitrogen percentage five times larger than that of the low protein line.

With the exception of the internode and grain from the high protein line, the lysine percentages varied significantly at the different harvest

Table 9. The nitrogen content of the leaf, leaf sheath, internode and grain of high and low protein corn plants at five stages of harvest expressed as a per cent of tissue dry weight

Harvest stage	Nitrogen per cent			
	Leaf	Leaf sheath	Internode	Grain
		Low protein		
I	2.76			
II	2.81	.76	.93	
III	2.58	.65	.53	1.42
IV	2.32	.54	.49	.96
V	1.61	.63	.44	.69
		High protein		
I	3.60			
II	3.62	1.52	2.70	
III	3.09	.87	3.07	3.53
IV	2.57	.97	2.07	3.66
V	1.48	.82	1.09	3.25

stages (Table 10). Lysine percentage did not decrease as consistently from Stages I to V as did nitrogen percentage (Table 11). The lysine percentage of the leaves of both lines remained constant until Stage V, and the internodes of both lines had low lysine percentages. At maturity the grain of the high protein line contained about three times as much lysine as the grain of the low protein line (0.38 and 0.14, respectively). This is in contrast to the fivefold diiferential for nitrogen percentage.

The leucine percentages were significantly different at the harvest stages in the leaves of both lines and in the grain of the low protein line

Table 10. Mean squares from analyses of variance of lysine percentages of leaves, internodes and grain of high and low protein corn harvested at five stages

Source of variation	Degrees of freedom	Mean squares	
		Low protein	High protein
Leaves			
Replications	3	.0084	.0105
Stages	4	.0443**	.1681**
Error	12	.0022	.0087
Internode			
Replications	3	.0003	.0023
Stages	3	.0012**	.0045
Error	9	.0001	.0016
Grain			
Replications	3	.0011	.0004
Stages	2	.0194**	.0035
Error	6	.0014	.0010

(Table 12). As with lysine, leucine percentage did not decrease much until Stage V. The leu ine percentages of the low protein line internodes were slightly lower than those of the high protein line, but in both lines the internode leucine percentage was considerably lower than the corresponding percentage in the leaves. As expected, the leucine percentage of the high protein grain at maturity (3.00 per cent) was much higher than that of the low protein line (.39 per cent).

Table 11. The mean lysine and leucine percentages of the leaf, internode and grain of high and low protein plants at five stages of harvest expressed as a per cent of tissue dry weight

Harvest stage	Lysine per cent			Leucine per cent		
	Leaf	Internode	Grain	Leaf	Internode	Grain
			Low protein			
I	.60			.86		
II	.68	.15		1.10	.15	
III	.63	.12	.28	.95	.15	.71
IV	.67	.12	.20	1.02	.17	.52
V	.42	.11	.14	.58	.13	.39
			High protein			
I	.85			1.18		
II	.71	.20		.97	.21	
III	.63	.22	.43	.97	.24	2.97
IV	.60	.25	.43	.86	.32	3.19
V	.30	.17	.38	.45	.23	3.00

Comparisons of high and low protein corn

Ratios of the per cents of nitrogen, leucine or lysine in each plant part in the high protein line to the corresponding per cents in the low protein line are given in Table 13. A ratio greater than one indicates a higher percentage in the high than in the low protein line. In some cases the ratios show considerable variation between stages, i.e., nitrogen in the internode. The ratios show no definite trends as the plant matures except in the grain where the ratios for all three compounds increase as the plants mature.

Table 12. Mean squares from analyses of variance of leucine percentages of leaves, internodes and grain of high and low protein corn harvested at five stages

Source of variation	Degrees of freedom	Mean squares	
		Low protein	High protein
Leaves			
Replications	3	.0427	.0845
Stages	4	.1587**	.2925**
Error	12	.0161	.0283
Internode			
Replications	3	.0008	.0009
Stages	3	.0010	.0083
Error	9	.0006	.0040
Grain			
Replications	3	.0043	.0832
Stages	2	.1017**	.0540
Error	6	.0088	.0414

With the exception of the leaf tissue at Stage V, all the ratios for nitrogen were greater than 1.0. Thus, selection for high nitrogen content in the grain also increased the nitrogen percentages of some of the vegetative tissues. The largest vegetative tissue difference between high and low protein lines was manifested in the internode which in the high protein line at Stage III contained 4.74 times as much nitrogen as the corresponding internode in the low protein line.

The ratios of the per cents for lysine and leucine in the two lines are lower than the corresponding ratios for nitrogen. For the leaves, the

Table 13. The ratios of nitrogen, lysine and leucine percentages in high protein lines to those in low protein lines for leaves, leaf sheaths, internodes and grain harvested at five stages

Harvest stage	Leaf	Leaf sheath	Internode	Grain
		Nitrogen		
I	1.30			
II	1.29	2.00	2.92	
III	1.20	1.33	5.74	2.49
IV	1.11	1.80	4.18	3.80
V	.92	1.30	2.48	4.72
		Lysine		
I	1.44			
II	1.04		1.36	
III	.99		1.94	1.52
IV	.90		2.06	2.15
V	.70		1.57	2.63
		Leucine		
I	1.37			
II	.88		1.36	
III	1.02		1.66	4.20
IV	.85		1.84	6.09
V	.77		1.71	7.70

ratios varied around 1.0, indicating little difference between the two corn lines for lysine and leucine percentages in the leaves. The internode ratios ranged between 1.0 and 2.0. The ratios for the mature grain were 2.63 for lysine and 7.70 for leucine which shows the disproportionate changes of the two amino acids associated with selection for high and low protein.

The ratios of leucine per cent to lysine per cent can be used as indicators of the quality of the corn protein (Table 14). A ratio of 1.0

Table 14. The ratios of leucine per cent/lysine per cent in the leaves, internodes and grain of high and low protein corn at five stages of harvest

Harvest date	Leaves	Internode	Grain
	Low protein		
I	1.45		
II	1.61	.99	
III	1.50	1.28	2.51
IV	1.52	1.41	2.62
V	1.38	1.21	2.71
	High protein		
I	1.38		
II	1.37	1.06	
III	1.55	1.09	6.94
IV	1.43	1.26	7.41
V	1.51	1.31	7.94

indicates equal amounts of lysine and leucine and thus well-balanced protein, whereas a high ratio indicates a deficiency of lysine.

In both high and low protein lines the leucine to lysine ratios of the leaves (1.38 - 1.61) and internodes (0.99 - 1.41) verify the observations that the protein of the vegetative tissue of corn is well balanced. On the other hand, the ratios of lysine to leucine in the grain of the low and high protein lines were 2.71 and 7.94, respectively. Obviously, this indicates

that the protein of the low nitrogen line was of better quality than that of the high protein line, but in neither line was the grain protein as good in quality as the leaf or internode protein.

The leucine/lysine ratio in the leaves and internode did not change appreciably in either line as the plants matured. Therefore, leucine probably is not preferentially translocated from the vegetative tissue to the grain.

DISCUSSION

The basis for this study was formed by two closely related problems: (a) the seed proteins of several economically important members of the grass family are deficient in the essential amino acid, lysine and (b) selection for higher protein often decreases the proportion of lysine in the grain protein. Any explanation found for the second problem can be extended to the first one.

The experiments were designed to relate the composition of the grain to that of the vegetative tissue and to the metabolism and translocation of lysine, leucine and nitrogen in and between the vegetative and seed tissues. Two corn lines representing extremes in protein content were analyzed for nitrogen and two essential amino acids--lysine which is deficient in the seed protein of corn and leucine which is not. It was postulated that by comparisons between the two lines and/or between the two amino acids some difference or differences would be apparent which could indicate the cause of lysine deficiency in the grain. In the light of the data obtained, four possibilities exist.

1. Leucine may be more effectively translocated from the vegetative tissue to the grain than is lysine. That is, relatively more lysine than leucine is left in the vegetative tissue at maturity. The consequence of this would be reflected by a decreasing leucine per cent/lysine per cent

ratio in the vegetative tissue as the plant matured. Such a trend was not found in either the high or low protein line.

Following similar reasoning it might be expected that when carbon-14 labelled lysine was injected into a plant and no radioactive lysine was recovered from the grain that the labelled lysine remained in the vegetative tissue. However, in the greenhouse experiment where carbon-14 labelled lysine was injected into high protein plants (treatment 3) no radioactive lysine was recovered from either the grain or the internodes.

Hence, the failure of lysine to be translocated from the vegetative to grain tissue does not appear to be the reason for its deficiency in the grain. Also, the possibility exists that lysine transport does not occur anywhere in the vegetative tissue after a certain stage in plant development.

2. Both amino acids may be translocated from the vegetative tissue but leucine is largely incorporated as such into the grain protein, whereas lysine undergoes metabolic conversion into other compounds or is used in respiration before, during or after translocation to the grain. This explanation seems to apply for the greenhouse experiment (treatments 1 and 3) where no labelled lysine could be

recovered in the grain of high protein plants. It is significant that the internodes of both lines gave labelling patterns similar to the grain for leucine and lysine. This indicates that lysine degradation in the high protein line may have occurred in the vegetative tissue and that enzymes responsible for amino acid synthesis might function mainly in the vegetative tissue and not in the grain.

Two other factors must be considered in evaluating this data: (a) The injection of labelled lysine and leucine into the internodes of high protein plants may have placed them--somewhat artificially--in an area of high protein content where zein amino acids were being synthesized and consequently lysine was degraded. (b) The labelled compounds were injected at a particular stage, i.e., 14 days after pollination, and the labelling pattern obtained could be characteristic for that stage and not necessarily all other stages.

The relative importance of lysine degradation contributing to the lysine deficiency in the grain could be assessed by determining the difference between lysine present in the entire plant at maximum vegetative growth and the amount present at maturity.

3. Nitrogen is available in some storage form before anthesis, and as the plant matures it is utilized mainly in the synthesis of zein amino acids. That the two lines are different in the nitrogen content of the vegetative tissue has been demonstrated in this study and by Hoerner and DeTurk (1938) who found at anthesis twice as much nitrogen in the vegetative tissue of the high as in the low protein line. The nature of the nitrogen-containing compounds is also important. The high protein per cent/low protein per cent ratios for nitrogen in all cases were higher than the corresponding ratios for lysine or leucine. Hence, the differences between the vegetative tissue of the two lines were due mainly to nitrogen-containing compounds other than lysine or leucine.

Stahmann (1963) pointed out that the leaves of different species show very few differences in protein composition. He postulated that regardless of the species the proteins have a specific role--to function in photosynthesis and leaf respiration--and hence their composition does not vary. There may be a certain "physiological" limit beyond which the quantity of proteins functional in the basic metabolism of the vegetative tissue cannot be increased. The small

differences in the leucine and lysine per cents between the vegetative tissue of the two lines support this idea. The additional nitrogen in the plant then would be shifted to some storage form--particularly in the internode (although not determined in this study, the so-called primary nitrogen acceptors--aspartic acid, glutamic acid and their amides--may act as nitrogen storage compounds). Then, after vegetative growth had ceased and the grain began to fill, the storage nitrogen could be utilized in the synthesis of zein amino acids. As mentioned before, the internodes of both lines gave labelling patterns similar to their grain with respect to lysine and leucine. This indicates that enzyme systems synthesizing leucine (and zein amino acids) may be active in the vegetative tissue. Little lysine synthesis during this grain filling period would be expected.

4. Another possibility is that nitrogen taken up by the plant from the soil after maximum vegetative growth is reached (or that contained in the roots and not measured in most studies) is utilized mainly for the production of zein amino acids. As much as 40 per cent of the nitrogen contained in the mature plant is taken up from the soil after anthesis (Hanway, 1960, and Hay et al., 1953). Since

little vegetative growth occurs after pollination this nitrogen would not be utilized in the synthesis of the amino acids and proteins for the grain. The data of Bressani and Conde (1961) and Zeleny (1935) who found that zein becomes an increasing proportion of the protein as the grain fills also suggest a decrease in the rate of lysine synthesis and an increase in the rate of zein amino acid synthesis.

It is difficult to assess the relative importance of the reasons discussed as contributory factors to lysine deficiency in the corn grain protein. The data in this study and others cited indicate that reasons 2, 3 and 4 or combinations of these are important considerations. It is recognized, however, that the problem is not solved but extended from the grain to the vegetative tissue. No basic genetic reasons or explanations as to why selection for high protein increases zein more than for other grain proteins are apparent from these data. Other important factors which were not included in this study contribute to the differences between the high and low protein lines, i.e., the ability of the roots to take up nitrate from the soil and subsequently reduce it to an amino form.

The method of introducing carbon-14 labelled compounds into the corn plant and after a period of several weeks collecting meaningful data from the grain was proven possible by the greenhouse experiment. The use or applications of the technique to study amino acid metabolism and protein

synthesis was obvious. There are, however, disadvantages in exact studies with corn. There is not a completely quantitative method of introducing radioactive materials into the corn plant. In this experiment it is quite likely that all the labelled leucine and lysine were not "effectively" introduced, i.e., all the radioactivity may not have been utilized normally in the plant metabolism. Also, since most chemical determinations allow for the analysis of very small amounts of material the size of the corn plant makes the determinations of total activities and the distribution of radioactivity difficult. The small grains for which more effective injection methods exist and which have smaller plant size would prove more useful when studying lysine and leucine metabolism per se. On the other hand, they do not have the gross genetic differences in protein content found in corn.

SUMMARY AND CONCLUSIONS

The purposes of this study were (a) to determine whether the vegetative tissue manifested the differences known to exist between Illinois high and low protein corn in the leucine and lysine content of the grain protein and (b) to determine if the changes of nitrogen, leucine and lysine content in the vegetative tissue as the plant matured could be related to the composition of the grain. Two experiments were run, one in the greenhouse during the winter of 1962-63 and one in the field during the summer of 1963.

In the greenhouse experiment, labelled leucine and lysine were injected into the internodes of high and low protein plants 14 days after pollination, and the distribution of radioactivity among 16 amino acids in the seeds and internodes at maturity was determined. When carbon-14 labelled lysine was injected into the high protein line, no radioactive lysine was found in either the grain or the second internode above the ear at maturity. However, when carbon-14 labelled lysine was introduced into low protein plants, considerable radioactive lysine was recovered in both the internode and grain. Radioactive leucine was found in the grain and internode of plants of both lines whenever introduced.

The recovery of large amounts of carbon-14 in isoleucine whenever radioactive leucine was injected indicated the possibility of a rather direct

pathway for the synthesis of isoleucine from leucine.

The use of carbon-14 labelled amino acids as a tool in long-term experiments studying amino acid metabolism and protein synthesis in corn was demonstrated.

In the field experiment, plants from the high and low lines were harvested at five stages: two weeks before pollination, at pollination, and two, four, and eight weeks after pollination. The leaf, leaf sheath and internode originating at the first node above the ear and the grain of plants from each stage were analyzed for nitrogen content. Lysine and leucine determinations were made on the leaf, internode and grain.

The major difference found in the vegetative tissue between the two lines was in the internode where in the high protein line it contained from 2 to 5 times as much nitrogen as the corresponding internode of the low protein line. The leaves and leaf sheaths of the high protein line had slightly higher nitrogen per cents than did the low line.

The difference between the two lines in the lysine and leucine content of the internode was not as large as the difference for nitrogen, and the leaves showed little or no difference at all.

The ratio of leucine to lysine per cent was calculated for the leaves, internodes and grain at different stages. Since this ratio did not change appreciably in the vegetative tissue as the plant matured, no differential translocation of leucine or lysine from the vegetative tissue was indicated.

Possible reasons for the disproportionate increases of leucine and lysine with increasing protein content were discussed.

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